This is the post print version of the article, which has been published in Genetic testing and molecular biomarkers . 2018, 22 (9),574-576. https://doi.org/10.1089/gtmb.2018.0118.

Association of desmin gene variant rs1058261 with cardiovascular disease, the TAMRISK study

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Running title: Desmin gene variant rs1058261 and cardiovascular disease

Abstract

Aims. Since desmin expression is diminished in vascular smooth muscle cells during reparative processes, we wanted to study whether a common intragenic single nucleotide polymorphism (SNP) at nucleotide position 828 (rs1058261) of the *DES* gene associates with hypertension, cerebrovascular complications, and all cardiovascular events in the Tampere adult population cardiovascular risk study (TAMRISK).

Materials and Methods. A Finnish periodic health examination cohort of 336 subjects with diagnosed hypertension and 473 controls was analyzed. Samples were genotyped for the polymorphism using TaqMan techniques. Prevalence of ischemic heart diseases, incidence of cerebrovascular diseases, and transient cerebral ischemic attacks (TIA) were followed up obtained by self-report and the National Hospital Discharge Registry (HILMO)

Results. There was no association of rs1058261 genotypes with hypertension at the age of 50. When the subjects were followed up to the age of 60, after adjustment for gender and BMI, subjects with genotype CC had higher incidence of cerebrovascular events (cerebrovascular diseases and TIA) (4.1%), compared to the T-allele (1.6%) (p=0.046). Also, those with genotype CC had higher incidence of all combined cardiovascular events (12.8%) compared to the subjects with the T-allele (8.5%) (p=0.028).

Conclusions. Our findings suggest that the variations in the *DES* gene may be involved in cardiovascular disease.

Keywords: Genetic variation, Intermediate Filaments/genetics, Desmin, Vascular diseases

Introduction

Desmin (DES) is a muscle-specific intermediate filament (IF) protein that connects cell organelles by forming a cytoskeletal network. It is expressed in skeletal, cardiac and smooth muscle cells (SMC) (Raguz et al. 1998, Fichna et al. 2014). Desmin is a useful marker for SMC differentiation (Nikkari et al. 1988). The only cells found in the media of normal human arteries are SMCs, responsible for arterial structure, function, and repair (Ross. 1999). In order to be able to meet the various requirements, the arterial SMC is capable of expressing different phenotypes, the extremes of which have been termed “contractile” and “synthetic”, based on their functional and morphological characteristics (Campbell and Campbell. 1990). A biochemical characterization of the SMC phenotypes has been achieved by studying the expression of muscle-related proteins (i.e. desmin and α-actin) in various states of smooth muscle (Allahverdian et al. 2018). In the majority of human atherosclerotic plaques, SMCs are desmin negative (Dartsch et al. 1989).

Desmin is encoded by the *DES* gene located on chromosome 2q35. *DES* encompasses nine exons within an 8.4-kb region and codes for 476 amino acids (Li et al. 1989). Since the recognition of the involvement of the *DES* gene in human disease in 1998 (Goldfarb et al. 1998), the number of publications on different *DES* mutations and associated phenotypes has increased steadily (van Spaendonck-Zwarts et al. 2011). We wanted to study whether a common intragenic single nucleotide polymorphism (SNP) at nucleotide position 828 (rs1058261) of the *DES* gene (Fichna et al. 2014) associates with hypertension, cerebrovascular complications and all combined cardiovascular events in a Finnish population from the Tampere adult population cardiovascular risk study (TAMRISK).

Materials and Methods

Subjects

TAMRISK study data was collected from periodic health examinations (PHE) done for 50-year-old men and women living in Tampere, a city in southern Finland with 220 000 inhabitants (Maatta et al. 2015). At the PHE in 2003 by a public health nurse, height (cm) and weight (kg) were recorded from which the body mass index (BMI) was calculated. Blood pressure measurement (mmHg) was done using a calibrated mercury sphygmomanometer. Serum glucose (mmoles/l), and total cholesterol, HDL-cholesterol, triglycerides (from which LDL-cholesterol was calculated by the Friedewald formula) were measured after an overnight fast by standard techniques. An interview was conducted using a structured questionnaire about health and health-related behavior. Buccal swabs for DNA extraction and a permissions form to use PHE information and national registry data were collected by mail separately of the physical examination during years 2006–2010. Using the patient's national identity code, data on hospitalizations including ICD-10 codes for discharge diagnoses were obtained from the National Hospital Discharge Registry (HILMO) maintained by the National Institute of Health and Welfare. Prevalence of ischemic heart diseases (I20-I25), incidence of cerebrovascular diseases (I60-I69), and transient cerebral ischemic attacks (TIAs) (G45) were followed up from 2005 to 2014 until the subjects were on the average 60 years old. In follow-up of the genotyped subjects, there were 67 with ischemic heart disease, 15 who had a diagnosis of cerebrovascular disease and 8 with TIA. The subjects with cerebrovascular disease and TIA were combined for the group with cerebrovascular events. All participants gave informed consent and the Ethics Committees of the Tampere University Hospital and the City of Tampere approved the study.

Cases (n=336) were subjects who had hypertension at the age of 50 years (as diagnosed by a physician) and for each case, at least one normotensive control subject (n=473) with

the same sex, and similar smoking habits, was chosen in order of admission from the PHE cohort (n=6000). The present study population at the age of 50 years thus included 809 subjects.

Genotyping

DNA was extracted from buccal swabs using a commercial kit (Qiagen Inc., Valencia, Calif., USA). An intragenic SNP at nucleotide position 828 (rs1058261) of the desmin gene was chosen (Fichna et al. 2014). For DNA genotyping, PCR was performed in a final volume of 5 µl containing 10 ng of sample DNA, 0.05 µl of custom SNP-specific Assay mix and 2.18 µl of Taqman Universal PCR Master Mix. The Assay mix used was C__11735969_20. Amplification proceeded for 40 cycles of 15 s at 95°C and 60 s at 60°C. Genotyping followed the Applied Biosystems (Pleasanton, CA, USA) protocol. Automatic genotype call was performed after PCR, by scanning plates on the 7900 HT Fast Real-Time PCR, which provides the SDS2.3 software (Applied Biosystems).

Statistical analysis

Logistic regression, one-way ANOVA or T-test for continuous variables and Chi-square test or Fisher's exact test for categorical variables were applied for the comparison of cases, controls and genotype groups. Analyses were carried out using SPSS 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Clinical characteristics of case group of 336 hypertensive subjects and 473 controls at the age of 50 years have been previously described (Kunnas et al. 2012). Samples were available and genotyping for rs1058261 was successful for 809 subjects: 336 cases and 473 controls (351 women and 458 men). The genotype frequencies were CC: 421

(52.0%), CT 329 (40.7%), and TT 59 (7.3%). The genotypes were in Hardy-Weinberg equilibrium (Chi-square=0.23, p>0.05).

There was no association of rs1058261 genotypes with hypertension when all genotypes were compared with each other (p=0.235); when CC genotype was compared to combined CT+TT genotypes (p=0.987); or when TT genotype was compared to combined CT+CC genotypes (p=0.102).

When the subjects were followed up to the age of 60, subjects with genotype CC had higher incidence of cerebrovascular events (4.0%), compared to the T-allele (1.5%) (p=0.031) (Table 1). When incidence of cerebrovascular events was adjusted by BMI and gender, the OR for CC genotype was 2.63 (p=0.046, CI 1.018 – 6.78), compared to T-allele carriers.

At the age of 60, those with genotype CC had also higher incidence of all combined cardiovascular events (12.6%) compared to the subjects with the T-allele (8.5%) (p=0.052) (Table 1). When adjusted by BMI and gender, the OR for CC genotype was 1.72 (p=0.028, CI 1.06-2.79) compared to T-allele carriers.

DES rs1058261 did not associate with other background characteristics of the study population (Table 1).

Discussion

The DES intragenic SNP (C>T) at nucleotide position 828 (rs1058261) is a synonymous codon that does not lead to amino acid change. However, it may be possible that rs1058261 is in linkage disequilibrium with a functional polymorphism that could lead to pathological changes at the protein level, since we report that this variation is associated with cerebrovascular complications and all combined cardiovascular events. At the age of

60, those with genotype CC had higher incidence of these events compared to subjects with the T-allele. However, there was no association of this SNP with hypertension. Previously, the C allele of the benign *DES* rs1058261 was associated with myofibrillar myopathies in two Polish families having other pathological mutations in the *DES* gene (Fichna et al. 2014). It is thought that intimal smooth muscle cells (SMCs) in native atherosclerotic plaque derive mainly from the medial arterial layer. Smooth muscle involvement is not reported as the main manifestation in *DES* related myopathy, but some manifestations that indicate smooth muscle involvement have been reported, like swallowing difficulties (Goldfarb et al. 1998).

Numerous research groups have used a variety of in vivo and in vitro model systems and clinical studies to demonstrate the conversion of normally contractile vascular SMC during phenotypic modulation to a less differentiated state, capable of proliferation, migration, and extracellular matrix secretion. These changes in vascular SMC phenotype are thought to underlie many vascular occlusive diseases (Nguyen et al. 2013). Phenotypic switching of the SMC plays a major role in a number of major diseases in humans including atherosclerosis and hypertension (Owens et al. 2004). Phenotype of the arterial smooth muscle cell in various stages of modulation or repair during fetal development, ageing, and atherosclerosis is characterized by “synthetic” SMCs that are rich in synthetic organelles and desmin-negative (Nikkari. 1991). Thus, variation in the expression of desmin may be considered important in the repair and function of the arterial wall. This may be influenced by the SNP *DES* rs1058261 since it was associated with cardiovascular events.

A weakness of the present study is the relatively small population, and few cases of cerebrovascular complications. Nevertheless, these cases were manifested at a relatively young age, with possible genetic predisposition. In conclusion, we report for the first time,

that the *DES* gene may be involved in cardiovascular disease as well, in addition to muscular disease (Goldfarb et al. 1998).

Acknowledgments

The expert technical assistance by Mirka Pietiläinen and Nina Peltonen is gratefully acknowledged. This study was supported by grants from Competitive research funding of the Pirkanmaa Hospital District.

Author Disclosure Statement

No competing financial interests exist.

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Table 1. Clinical characteristics (means ± SD) of the study population stratified according to *DES* rs1058261 genotypes.

	CC (421)	CT (329)	TT (59)	P value * CC vs. CT vs. TT	P value * CC vs. (CT+TT)	P value * TT vs. (CC +CT)	P value ** CC vs. (CT+TT)
Cerebrovascular events % (n)	4.0 (17)	1.5 (5)	1.7 (1)	0.097	**0.031**	1.000	**0.046**
All combined cardiovascular events % (n)	12.6 (53)	8.2 (27)	10.2 (6)	0.138	0.052	0.881	**0.028**
Body mass index kg/m^2 (SD)	26.7 (4.5)	26.6 (4.4)	26.5 (4.7)	0.868	0.604	0.790	
Glucose, mmol/L (SD)	5.03 (1.13)	5.00 (1.18)	4.93 (0.90)	0.810	0.620	0.585	
Cholesterol, mmol/L (SD)	5.39 (0.99)	5.40 (0.99)	5.47 (0.83)	0.823	0.695	0.563	
Systolic blood pressure, mmHg (SD)	135.5 (17.7)	133.7 (16.3)	135.4 (17.8)	0.370	0.219	0.766	
Diastolic blood pressure, mmHg (SD)	87.6 (10.5)	87.6 (9.3)	89.1 (11.4)	0.553	0.834	0.279	

SD, standard deviation. * Chi-square test, Fisher's exact test, One-way ANOVA or T-test. ** Logistic regression adjusted by BMI and gender. P values <0.05 are in bold.